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ENZYMATIC TEST KIT FOR THE DETERMINATION OF D-GLUCOSE AND D-FRUCTOSE IN GRAPE JUICE AND WINE

PRODUCT

Product no. 4A140, **for 30 tests, for *in vitro* use only.**

PRINCIPLE OF MEASUREMENT

Glucose and fructose are the main sugars found in grape juice and wine and are determined enzymatically according to the following equations:

$$\text{Glucose} + \text{ATP} \xrightarrow{\text{HK}} \text{Glucose-6-phosphate} + \text{ADP}$$
$$\text{Fructose} + \text{ATP} \xrightarrow{} \text{Fructose-6-phosphate} + \text{ADP}$$

Glucose and fructose react with adenosine triphosphate (ATP) in the presence of the enzyme hexokinase (HK) to form glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P).

$$\text{G6P} + \text{NADP}^+ \xrightarrow{\text{G6PDH}} \text{Gluconate-6-phosphate} + \text{NADPH} + \text{H}^+$$

G6P is oxidised by nicotinamide adenine dinucleotide phosphate (NADP) to gluconate 6-phosphate using glucose-6-phosphate dehydrogenase (G6PDH) enzyme as a catalyst. The amount of NADPH formed is measured at 340 nm and is stoichiometrically related to the amount of glucose consumed.

$$\text{Fructose-6-phosphate} \xleftrightarrow{\text{PGI}} \text{Glucose-6-phosphate}$$

Next, the enzyme phosphoglucose isomerase (PGI) is added to convert the F6P to G6P. The G6P now formed reacts with NADP and the NADPH determined is stoichiometrically related to the amount of fructose in the sample.

CONTENTS

The kit includes the following reagents:

Reagent No.	Reagent	Preparation	Quantity	Stability
1	Buffer	To activate the Buffer, add the contents of Reagent No.2	33 mL	18 months at 4°C (6 months once activated)
2	Coenzymes (ATP/NADP)	Coenzymes (ATP/NADP) and mix with inversion until completely dissolved.	0.2 g	18 months at 4°C
3	G6PDH/HK	Swirl gently before use	0.7 mL	18 months at 4°C
4	PGI	Swirl gently before use	0.7 mL	18 months at 4°C
5	Standard	Nil	3.3 mL	18 months at 4°C

The shelf life of Reagents 1 & 2 can be extended by placing aliquots in a freezer.
Do not freeze enzyme reagents 3 & 4. Failure to store reagents at the recommended temperature will significantly reduce their shelf life. For concentration of the Standard, refer to the label on the bottle.

SAFETY

- **Wear safety glasses**
- **Do not ingest Buffer or Standard as they contain sodium azide as a stabilizer**

PROCEDURE

Operating Parameters

Wavelength	340 nm
Cuvettes	1cm, quartz, silica, methacrylate or polystyrene
Temperature	20 – 25˚C
Final volume in cuvette	3.04 mL
Zero	against air without cuvette in light path

SAMPLE PREPARATION

Samples should be diluted with distilled water to ensure that the concentration in the assay solution is no more than 1.0 g/L. For the majority of dry wine samples, a 1 in 10 dilution is satisfactory.
Semi-sweet wines may require up to a 1 in 50 dilution, while fortified and dessert wines may require up to a 1 in 100 dilution or greater. As a general guide, further dilution is required if the final A_3 absorbance reading is greater than 1.2 absorbance units. Samples may be used directly without decolourisation.
Turbid samples must be either centrifuged or filtered through Whatman No. 1 filter paper to clarify.

SAMPLE ANALYSIS

a. Check that Reagent No.1 Buffer has been activated by the addition of Reagent No.2 Coenzymes
b. Pipette the following volumes of reagents into the cuvettes:

Reagent	Blank assay	Standard assay	Samples
1. Buffer/Coenzyme mix	1.00 mL (1000 µL)	1.00 mL (1000 µL)	1.00 mL (1000 µL)
Distilled water	2.00 mL (2000 µL)	1.90 mL (1900 µL)	1.90 mL (1900 µL)
Sample or Standard		0.10 mL (100 µL)	0.10 mL (100 µL)

c. Mix well and read absorbances, A_1, after 3 minutes.
d. Pipette the following reagent into the cuvettes:

3. G6PDH/HK	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)

e. Mix well and read absorbances, A_2, after 10 minutes.
f. Pipette the following reagent into the cuvettes:

4. PGI	0.02 mL (20µL)	0.02 mL (20µL)	0.02 mL (20µL)

g. Mix well and read absorbances, A_3, after 10 minutes.

CALCULATIONS*

1. Calculate the Corrected Absorbance for the sample for D-Glucose:

$$\text{D-Glucose Absorbance, } A_G = (A_2 - A_1) - (\text{Blank}A_2 - \text{Blank}A_1)$$

2. Calculate the D-Glucose concentration as follows:

$$\text{D-Glucose Concentration (g/L)} = A_G \times 0.8637 \times \text{Dilution Factor}$$

3. Calculate the Corrected Absorbance for the sample for D-Fructose:

$$\text{D-Fructose Absorbance, } A_F = (A_3 - A_2) - (\text{Blank}A_3 - \text{Blank}A_2)$$

4. Calculate the D-Fructose concentration as follows:

$$\text{D-Fructose Concentration (g/L)} = A_F \times 0.8694 \times \text{Dilution Factor}$$

5. Add the D-Glucose and D-Fructose results together to get the total residual sugar concentration

6. Do the same for the Standard by substituting the Standard absorbance values in place of the sample absorbance values.

*A calculation spreadsheet is available for download at:
http://www.vintessential.com.au/certification/calculation-worksheets/

7. Precision (where x is the D-glucose or D-fructose concentration in the sample in g/l):

Repeatability $r = 0.056x$ *Reproducibility* $R = 0.12 + 0.076x$

REFERENCES

1. “Compendium of International Methods of Wine and Must Analysis” OIV, Vol 1, 2006, MA-E-AS311-02-GLUFRU5, p4.